REMARKS

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow.

Claims 2, 22, and 23 are requested to be cancelled without prejudice to further prosecution on the merits.

Claims 1, 5-8, and 24 are currently being amended. Support for amended claims 1, 5-8, and 24 may be found in the original claims and specification as filed.

This amendment adds, changes and/or deletes claims in this application. A detailed listing of all claims that are, or were, in the application, irrespective of whether the claim(s) remain under examination in the application, is presented, with an appropriate defined status identifier.

After amending the claims as set forth above, claims 1, 3-9, 13, and 24 are now pending in this application.

35 U.S.C. § 102

In the Office Action dated July 22, 2004 and in the Advisory Action dated February 1, 2005, the Examiner maintained the rejection of claims 1, 2, 4-9, and 17 under 35 U.S.C. § 102(b) as being anticipated by Kigawa *et al.* (WO 98/08975), as evidenced by Sena *et al.* (U.S. Patent No. 5,670,316). Applicants respectfully traverse the rejection for the following reasons.

First, Applicants note that claim 1, from which all other pending claims depend, has been amended to recite:

"***RecA***" instead of "RecA-like recombinase";

"***ATPγS***" instead of "a non-hydrolyzable nucleotide co-factor"; and

"wherein the number of ATPγS molecules is...***5 times or less*** than the number of RecA molecules" instead of "wherein the number of ATPγS molecules is...10 times or less than the number of RecA molecules" (Emphasis added.)

Amended as such, Applicants respectfully contend that the claims are not anticipated by Kigawa *et al.* The numeric ranges presented in Kigawa *et al.* (page 14, lines 15 to 28 and page 17, lines 2 to 5), are derived from information presented in the list of references cited by Kigawa *et al.* (*See* references cited at Kigawa *et al.*, page 14, lines 17 to 24). However, Kigawa *et al.* merely recite concentration ranges of RecA and ATPγS ***that might possibly be used in a reaction***. Simply citing these ranges is not an indication that a RecA/single-stranded nucleic acid probe complex can be efficiently prepared with a high yield by arbitrarily combining a potential concentration of ATPγS, a potential concentration of RecA, and a potential concentration of nucleic acid probe.

The Examiner asserted that the claims are anticipated by selecting particular values included within the ranges disclosed by Kigawa *et al.* For instance, the Examiner combined the lowest concentration of nonhydrolyzable nucleotide cofactor (0.01 mM ATPγS) and the highest concentration of RecA-like recombinase (0.025 mM RecA protein), as disclosed by Kigawa *et al.* (page 14, lines 15 to 28 and page 17, lines 2 to 5). Taken as such, the Examiner calculated the ratio "ATPγS/RecA protein = 0.4." The Examiner then concluded that this value is formally included in the range recited in the pending claims, and hence rejected the claims as anticipated by Kigawa *et al.*

However, by combining the highest concentration of ATPγS disclosed by Kigawa *et al.* (3 mM ATPγS) and the lowest concentration of RecA (0.002 mM RecA protein), a ratio of 1500 is achieved, which is outside of the claimed range. Selecting concentrations as such, Kigawa *et al.* can be argued to disclose ratios of ATPγS and RecA that range from 0.4 to 1500. The pending claims recite a ratio of ATPγS and RecA that is 5 or less. Therefore, selecting concentrations as such, Kigawa *et al.*'s disclosed range can only, at best, be argued to overlap the recited range.

Under MPEP § 2131.03:

> When the prior art discloses a range which touches, overlaps or is within the claimed range, but no specific examples falling within the claimed range are disclosed, a case by case determination must be made as to anticipation. In order to anticipate the claims, the claimed subject matter must be disclosed in the reference with "***sufficient specificity to constitute an anticipation under the statute***." What constitutes a "sufficient specificity" is fact dependent. ***If the claims are directed to a narrow range, the reference teaches a broad range***, and ***there is evidence of unexpected results within the claimed narrow range***, depending on the other facts of the case, ***it may be reasonable to conclude that the narrow range is not disclosed with "sufficient specificity" to constitute an anticipation of the claims. The unexpected results may also render the claims unobvious***. (*Emphasis added*).

Applicants respectfully assert that Kigawa *et al.* does not teach a ***specific*** RecA/single-stranded nucleic acid probe complex in which the ratio of ATPγS to RecA is 5 or less ***at all***, let alone with "***sufficient specificity***."

As noted above, Kigawa *et al.*'s disclosed range can only, at best, be argued to overlap the claimed ranges. Further, the ranges and ratios described above are not ***specific*** examples. The Examiner previously asserted that "specific reaction mixture, No. 23 (Table 1, page 26)" anticipates or renders obvious ranges in which the ratio of non-hydrolyzable nucleotide co-factor to RecA is 5 or less, or even in which the ratio of non-hydrolyzable nucleotide co-factor to RecA is 3 or less. As described by the Examiner, specific reaction mixture No. 23 includes 1 ng of 275 bp homologous probe, 500 ng of λ DNA fragments, 15 μg of RecA, and 0.3 mM GTPγS in a 9 μl reaction volume. As such, the Examiner asserted that "the molar ratio of GTPγS to RecA is 6.8."

Applicants respectfully assert that specific reaction mixture No. 23 does not anticipate the claims as amended. The pending claims recite "RecA" not "RecA-like recombinase"; "ATPγS" not "GTPγS"; and "wherein the number of ATPγS molecules is...5 times or less than the number of RecA molecules" not "wherein the number of ATPγS molecules is...10 times or less than the number of RecA molecules."

Applicants also assert that Kigawa *et al.*, including specific reaction mixture No. 23, does not suggest the claims as amended. Applicants contend that the technical implications of using ATPγS as a RecA substrate are different than using GTPγS as a RecA substrate.

For example, RecA has the ability to hydrolyze ATP (to ADP + Pi) but cannot hydrolyze GTP (to GDP + Pi).

Furthermore, the results for reaction mixture No. 22 and No. 23 in Kigawa *et al.* demonstrate that ***decreasing*** the ratio of GTPγS/RecA ***decreases*** specificity. (*See* Kigawa *et al.*, Table 2 on pages 26 and 27, results for reaction mixtures No. 22 and No. 23). As noted by the Examiner, reaction mixture No. 22 has a GTPγS/RecA ratio of 13.6/1 and reaction mixture No. 23 has a GTPγS/RecA ratio of 6.8/1. The decrease in the GTPγS/RecA ratio for reaction mixture No. 23 results in a decrease in percentage specificity. (The percentage specificities for reaction mixture No. 22 and No. 23 are 78.8% and 60.3%, respectively, *see* Kigawa *et al.*, Table 2 on pages 26 and 27). Therefore, Kigawa *et al.* teaches away from using a lower ratio of GTPγS/RecA. Applicants respectfully contend that Kigawa *et al.* does not include a specific example that anticipates the claimed ranges nor does Kigawa *et al.* suggest the claimed ranges.

Moreover, using ratios as recited in the claimed ranges, Applicants have observed unexpected results. At the time of filing, from the point of view of reaction efficiency, the reaction was commonly performed with a large excess of ATPγS relative to RecA and nucleotides. As such, the concept of improving reaction efficiency by reducing the amount of ATPγS in the reaction system was unexpected, and in fact, was contrary to common practice. In particular, one skilled in the art would not expect that using ratios of ATPγS relative to RecA of 5 or less would improve reaction efficiency. For these reasons, Applicants respectfully contend that the claims are not anticipated or made obvious by the teachings of Kigawa *et al.* as evidenced by Sena *et al.*

In responding to Applicants assertion of "unexpected results," the Examiner noted that "Applicants presented them only for RecA, and the claims are drawn to any recombinase." Applicants note that the claims have been amended to recite "RecA."

The Examiner also noted that "the results of specificity determination were not presented for mixtures A and B at zero salt concentration." Applicants respectfully assert that it is not necessary to observe the results of specificity determination for mixtures A and B at zero salt concentration to ascertain that the disclosed results are unexpected. Reaction mixtures A and B have identical ratios of ATPγS/nucleotides (*i.e.*, 1/5). However, reaction mixture A has a ***higher*** ratio of ATPγS/RecA (*i.e.*, 1/1) than reaction mixture B (*i.e.*, 1/2), and reaction mixture A has a ***lower*** specificity in 100 mM NaCl (*i.e.*, 37.8%) than reaction mixture B in 100 mM NaCl (*i.e.*, 55.7%). At the time of filing, one skilled in the art would expect that the specificity would be ***higher*** for a reaction mixture with a ***higher*** ratio of ATPγS/RecA at identical salt concentrations. This result is unexpected.

The Examiner also asserted that "the mixture for which supposedly unexpected results were obtained and shown at zero salt (mixtures C, E, G, H, I, J, K and L) had both the ATPγS/nucleotides and ATPγS/RecA ratios varied, therefore it is not clear whether lowering the ATPγS/RecA ratio is truly the factor solely responsible for the observed results." Applicants respectfully assert that, regardless of whether "lowering the ATPγS/RecA ratio" is ***solely*** responsible for the observed results, the Examiner cannot deny that the results demonstrate that "***lowering*** the ATPγS/RecA ratio" contributes to an ***increased*** reaction specificity and that this result is unexpected based on general knowledge in the art.

Furthermore, the specification includes experiments in which the ratio of ATPγS/nucleotides was held constant while the ratio of ATPγS/RecA was varied. These experiments show, unexpectedly, that decreasing the ratio of ATPγS/RecA improved the reaction specificity. For example, mixtures A, C, E, and F have identical ratios of ATPγS/RecA (*i.e.*, 1/1) but increasing ratios of ATPγS/nucleotides (*i.e.*, 1/5, 1/4, 1/3, and 1/2, respectively). By increasing the ratios of ATPγS/nucleotides from 1/5 to 1/4, 1/5 to 1/3, or 1/5 to 1/2, the specificity in 100 mM NaCl is increased from 37.8% (mixture A) to 83.6% (mixture C); to 87.5% (mixture E); or to 84.8% (mixture F). Likewise, mixtures B and D have identical ratios of ATPγS/RecA (*i.e.*, 1/2) but increasing ratios of ATPγS/nucleotides (*i.e.*, 1/5 and 1/4, respectively). By increasing the ratios of ATPγS/nucleotides from 1/5 to 1/4, the specificity in 100 mM NaCl is increased from 55.7% (mixture B) to 80.5% (mixture D). These results are unexpected.

Moreover, assuming that the Examiner is correct (*i.e.*, "that it is not clear whether lowering the ATPγS/RecA ratio is truly the factor ***solely*** responsible for the observed results"), one must assume that the ***increasing*** ratio of ATPγS/nucleotides in reaction mixtures C, E, G, H, I, J, K and L must be at least partially responsible for the observed ***decreasing trend*** in specificity percentages for these same reaction mixtures. This is contrary to generally knowledge in the art and to the results provided in the specification. Because only a minimal effect on specificity is observed when the ATPγS/nucleotides ratio is increased from 1/4 to 1/2 (*e.g.*, comparing the results for mixtures C, E, and F), the most reasonable interpretation of these results is that a threshold concentration of ATPγS relative to nucleotides is required in which the ratio of ATPγS/nucleotides is 1/4 or higher. Applicants note that it is generally understood by those of ordinary skill in the art that RecA protein generally binds to one ATP (or ATPγS) in a 1:1 ratio, and that RecA protein generally binds to a nucleotide in a 1:3 (or 1:4) ratio. As such, the minimum number of ATPγS molecules relative to nucleotides that are required for an efficient reaction with high yield (*i.e.*, "one quarter or more than the number of molecules of nucleotide residues") may be derived from facts already known in the art.

The specification also includes experiments in which the concentration of RecA was held constant while the concentration of ATPγS was increased. For example, in reaction mixtures E, G, H, I, J, K, and L, the concentration of single-stranded probe (30 μM) and RecA (10 μM) remain constant while the concentration of ATPγS is increased (10 μM, 20 μM, 50 μM, 100 μM, 300 μM, 500 μM, and 2000 μM, respectively). These reaction mixtures display decreasing specificity percentages as the concentration of ATPγS is increased, either in 0 mM NaCl or in the presence of 100 mM NaCl. (*See* Table 2, page 25, results for mixtures E, G, H, I, J, K, and L in 0 mM NaCl and in the presence of 100 mM NaCl). Taken as a whole, the experiments disclosed in the specification refute the generally accepted concept in the art that a large excess of ATPγS is required to improve reaction efficiency and yield. These results are unexpected.

Finally, the Examiner asserted:

> the results of reaction specificity for reaction mixtures A-K obtained with 100 mM NaCl neutralizes differences arising from varying the ratios of ATPγS/nucleotides and ATPγS/RecA. Therefore, the unexpected results, if present at

> all, are certainly not visible at 100 mM NaCl, and would not apply to any reaction conditions.

Applicants respectfully disagree.

The results provided in the specification clearly indicate that the claimed ATPγS/RecA ratios and ATPγS/nucleotides ratios provide superior results compared to ratios outside the claim scope, irrespective of salt concentrations. Reaction conditions C to H, (as shown in Table 1, page 24), all fall within the scope of the claimed ATPγS/RecA ratios and ATPγS/nucleotides ratios. Table 2, page 25 compares the results obtained under reaction conditions C to H (at either 0 mM NaCl or 100 mM NaCl), to the results obtained under reaction conditions I to L (at either 0 mM NaCl or 100 mM NaCl), which are outside of the scope of the claimed ATPγS/RecA ratios and ATPγS/nucleotides ratios. These results clearly indicate that reaction conditions C to H provide superior specificity to reaction conditions I to L at identical salt concentrations (*i.e.*, at either 0 mM NaCl or 100 mM NaCl).

While the results show that the presence of salt can increase the reaction efficiency and yield, the results also show that the presence of salt cannot overcome the deficiency of not having the preferred ratios of ATPγS/RecA and ATPγS/nucleotides. For example, in 100 mM NaCl, mixtures J, K, and L do not display specificity percentages higher than 33%. Conversely, in the absence of NaCl, mixtures C and E display specificity percentages higher than 67%. Further, mixtures E, G, H, I, J, K, and L display the same trend of decreasing specificity percentages either in 0 mM NaCl or in 100 mM NaCl. (*See* Table 2, page 25). These results clearly indicate that the claimed ratios of ATPγS/RecA and ATPγS/nucleotides are important to reaction specificity at various salt concentrations.

Applicants respectfully contend that the observed results discussed above are not random and are unexpected. Therefore, Applicants request that the Examiner reconsider and withdraw the rejection of claims 1, 2, 4-9, and 17 under 35 U.S.C. § 102(b) as being anticipated by Kigawa *et al.* as evidenced by Sena *et al.*

35 U.S.C. § 103

In the Office Action, the Examiner maintained the rejection of claim 3 under 35 U.S.C. § 103, as being unpatentable over Kigawa *et al.* and Sena *et al.* The Examiner also maintained the rejection of claim 13 under 35 U.S.C. § 103 as being unpatentable over Kigawa *et al.* and Kigawa-2 *et al.* (EP 0 687 738 A1). Further, the Examiner rejected claim 24 under 35 U.S.C. § 103(a) as being unpatentable over Kigawa *et al.* Applicants respectfully traverse the rejection for the following reasons.

Claims 3, 13, and 24 depend from claim 1 and include the limitation, "wherein the number of ***ATPγS molecules*** is...***5 times or less*** than the number of ***RecA molecules***." For the reasons discussed above, Applicants respectfully contend that none of the cited references, alone or in combination, teach or suggest all the limitation of claim 3, 13, or 24. Further, as noted above, Applicants contend that the claimed ratios provide unexpected results. As such, Applicants respectfully request that the Examiner reconsider and withdraw the rejection of claims 3, 13, and 24 under 35 U.S.C. § 103.

Applicants believe that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested. The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, applicants hereby petition for such extension under 37 C.F.R. § 1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

Date 3/9/2005

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